

AMENDMENTS TO THE CLAIMS

Listing of Claims:

This Listing of Claims will replace all prior versions, and listings, of claims in the application:

1-17 (Cancelled)

18. (Currently amended) An apparatus comprising:

- a) a reaction chamber containing a template comprising ~~an unknown~~ a nucleic acid molecule attached to an immobilization surface;
- b) an inlet channel in fluid communication with the reaction chamber;
- c) an outlet channel in fluid communication with the reaction chamber;
wherein the ~~first-inlet~~ and ~~second-outlet~~ channels are distinct and separate from the reaction chamber and positioned before and after the reaction chamber;
- d) a first Raman detection unit operably coupled to the inlet channel and configured to perform surface enhanced Raman spectroscopy (SERS); and
- e) a second Raman detection unit operably coupled to the outlet channel at a location outside the reaction chamber and configured to perform surface enhanced Raman spectroscopy (SERS), ~~and~~
- f) a mesh inside the outlet channel;
- a first concentration of nucleotide in the inlet channel of the reaction chamber; and
- a second concentration of the nucleotide in the outlet channel of the reaction chamber;

wherein the second Raman detection unit is configured to measure the concentrations of nucleotides by Raman spectroscopy at a location outside the reaction chamber as the nucleotides flow through the outlet channel;

wherein the first and second Raman detection units are distinct and separate from the reaction chamber and are positioned before and after the reaction chamber; and

wherein a difference between the first concentration of nucleotide and the second concentration of nucleotide is proportional to the amount of nucleotide incorporated into a newly synthesized strand complementary to the nucleic acid.

wherein the first or second Raman detection unit is capable of detecting at least one unlabeled nucleotide at a single nucleotide level.

19. (Canceled)

20. (Currently amended) The apparatus of claim 18, wherein the concentrations of nucleotides ~~is~~are measured by Raman spectroscopy as they flow through the inlet channel and outlet channel.

21. (Previously Presented) The apparatus of claim 18, further comprising SERS active metal nanoparticles in the inlet channel and outlet channel.

22. (Previously Presented) The apparatus of claim 18, wherein the inlet channel and outlet channel diameter is between about 100 and about 200 micrometers in diameter.

23. (Currently amended) The apparatus of claim 18, ~~further comprising a mesh inside the outlet channel, the mesh comprising wherein the mesh comprises~~ silver, gold, platinum, copper or aluminum.

24-35 (Cancelled)

36. (Currently amended) An apparatus comprising:

- a) a reaction chamber containing a template comprising ~~an unknown~~ nucleic acid molecule attached to an immobilization surface;
- b) an inlet channel in fluid communication with the reaction chamber;
- c) an outlet channel in fluid communication with the reaction chamber;
~~wherein the first inlet and second outlet channels are distinct and separate from the reaction chamber and positioned before and after the reaction chamber;~~
- d) a first Raman detection unit operably coupled to the inlet channel, wherein surface enhanced Raman spectroscopy (SERS) active particles are in the inlet channel; ~~and~~
- e) a second Raman detection unit operably coupled to the outlet channel at a location outside the reaction chamber, wherein SERS active particles are in the outlet channel, ~~and~~
~~f) a mesh inside the outlet channel,~~
~~wherein the second Raman detection unit is configured to measure the concentrations of nucleotides by Raman spectroscopy at a location outside the reaction chamber as the nucleotides flow through the outlet channel;~~

wherein the first and second Raman detection units are distinct and separate from the reaction chamber and are positioned before and after the reaction chamber; and

wherein a difference between a first concentration of nucleotide in the inlet channel and a second concentration of nucleotide in the outlet channel is proportional to the amount of nucleotide incorporated into a newly synthesized strand complementary to the nucleic acid molecule in the reaction chamber,

wherein the first or second Raman detection unit is capable of detecting at least one unlabeled nucleotide at a single nucleotide level.

37. (Canceled)

38. (Currently amended) The apparatus of claim 36, wherein the concentrations of nucleotides ~~is~~are measured by Raman spectroscopy as they flow through the inlet channel and outlet channel.

39. (Previously Presented) The apparatus of claim 36, wherein the inlet channel and outlet channel diameter is between about 100 and about 200 micrometers in diameter.

40. (Previously Presented) The apparatus of claim 36, further comprising a mesh inside the outlet channel, the mesh comprising silver, gold, platinum, copper or aluminum.

41. (Currently amended) An apparatus comprising:

- a) a reaction chamber containing a template comprising ~~an unknown~~^a nucleic acid molecule attached to an immobilization surface;
- b) an inlet channel in fluid communication with the reaction chamber;
- c) an outlet channel in fluid communication with the reaction chamber;
- wherein the ~~first~~inlet and ~~second~~outlet channels are distinct and separate from the reaction chamber and positioned before and after the reaction chamber; and
- d) a first Raman detection unit operably coupled only to the ~~outlet~~inlet channel at a location outside the reaction chamber and configured to perform surface enhanced Raman spectroscopy (SERS), and
- e) a mesh inside the outlet channel;
- wherein the ~~second~~ second Raman detection unit is configured to measure the concentrations of nucleotides by Raman spectroscopy at a location outside the reaction chamber as the nucleotides flow through the outlet channel;
- wherein the second Raman detection unit is distinct and separate from the reaction chamber and is positioned after the reaction chamber;
- wherein a difference in nucleotide concentration measured with the first Raman detection unit and the second Raman detection unit is proportional to the amount of nucleotide that has been incorporated into a newly synthesized strand complementary to the nucleic acid,
- wherein the first or second Raman detection unit is capable of detecting at least one unlabeled nucleotide at a single nucleotide level.

42. (Canceled)

43. (Currently amended) The apparatus of claim 41, wherein the concentrations of nucleotides ~~is~~are measured by Raman spectroscopy as they~~the~~flow through the inlet channel and outlet channel.

44. (Previously Presented) The apparatus of claim 41, wherein the inlet channel and outlet channel diameter is between about 100 and about 200 micrometers in diameter.

45. (Currently amended) An apparatus comprising:

- a) a reaction chamber containing a template comprising ~~an unknown~~a nucleic acid molecule attached to an immobilization surface;
- b) an inlet channel in fluid communication with the reaction chamber;
- c) an outlet channel in fluid communication with the reaction chamber;
wherein the ~~first~~inlet and ~~second~~outlet channels are distinct and separate from the reaction chamber and positioned before and after the reaction chamber;
- d) a first Raman detection unit operably coupled only to the ~~outlet~~inlet channel at a location outside the reaction chamber, wherein SERS active particles are in the outlet channel, and
f) a mesh inside the outlet channel;
wherein ~~the~~a second Raman detection unit is configured to measure the concentrations of nucleotides by Raman spectroscopy at a location outside the reaction chamber as the nucleotides flow through the outlet channel;

wherein the second Raman detection unit is distinct and separate from the reaction chamber and is positioned after the reaction chamber;

wherein a difference in nucleotide concentration is proportional to the amount of nucleotide that has been incorporated into a newly synthesized strand complementary to the nucleic acid,

wherein the first or second Raman detection unit is capable of detecting at least one unlabeled nucleotide at a single nucleotide level.

46. (Canceled)

47. (Currently amended) The apparatus of claim 45, wherein the concentrations of nucleotides ~~is~~are measured by Raman spectroscopy as the nucleotides flow through the outlet channel.

48. (Previously Presented) The apparatus of claim 45, wherein the inlet channel and outlet channel diameter is between about 100 and about 200 micrometers in diameter.

49. (Previously Presented) The apparatus of claim 18, wherein the reaction chamber further comprises a primer and a polymerase, and wherein the template, the primer and the polymerase are confined to the reaction chamber.

50. (Previously Presented) The apparatus of claim 36, wherein the reaction chamber further comprises a primer and a polymerase, and wherein the template, the primer and the polymerase are confined to the reaction chamber.

51. (Previously Presented) The apparatus of claim 41, wherein the reaction chamber further comprises a primer and a polymerase, and wherein the template, the primer and the polymerase are confined to the reaction chamber.

52. (Previously Presented) The apparatus of claim 45, wherein the reaction chamber further comprises a primer and a polymerase, and wherein the template, the primer and the polymerase are confined to the reaction chamber.

53. (New) An apparatus comprising:
a) a reaction chamber containing a template comprising a nucleic acid molecule attached to an immobilization surface;
b) an inlet channel in fluid communication with the reaction chamber;
c) an outlet channel in fluid communication with the reaction chamber;
wherein the inlet and outlet channels are distinct and separate from the reaction chamber and positioned before and after the reaction chamber;
d) a first Raman detection unit operably coupled to the reaction chamber, wherein the Raman detection unit is arranged to detect nucleotide concentrations in the reaction chamber without background signals from the nucleic acid,

wherein the first or second Raman detection unit is capable of detecting at least one unlabeled nucleotide at a single nucleotide level.

54. (New) The apparatus of claim 53, further comprising a second Raman detection unit operably coupled to the inlet channel.

55. (New) The apparatus of claim 53, further comprising a mixer operably coupled to the reaction chamber.